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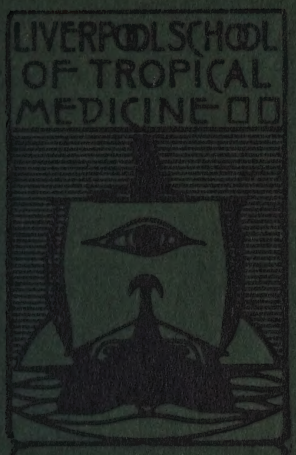

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Factors in the Transmission and Prevention of Malaria in the Panama Canal Zone

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FACTORS IN THE TRANSMISSION AND PREVENTION OF MALARIA IN THE PANAMA CANAL ZONE

BY

S. T. DARLING, M.D.

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INTRODUCTION

In every malarial region it is important that the species of mosquitos common to that region should be recognised, their breeding habits should be studied, and a determination made of the species of Anophelines, hospitable to malaria, and those transmitting it. The English observers, Stephens and Christophers, noticed that certain species of Anophelines were natural transmitters of malarial fever, while others were rarely, if ever, found infected naturally, although it was possible to infect them under laboratory conditions. We know that the breeding habits of Anophelines vary, too, considerably, and it may be said that there is as much selection of breeding places by Anophelines as there is selection of feeding grounds by fish. Trout, salmon, and bull-heads have their analogues among Anopheline larvae; some of the latter requiring fresh aerated water, or water containing much green algae. Others are found in tree-holes and recesses of epiphytic tree plants, such as Bromelias, where they prey upon other species, while others preferring fresh aerated water are so adaptable that they will flourish in sewage streams, or in brackish water containing half its volume of sea water.

Some species require an abundance of sunlight, while other sylvan species prefer shady pools in which chlorophyll-bearing algae are relatively absent. The Anophelines insusceptible to malaria may be more limited in their choice of breeding places, so that in the work of malarial mosquito destruction the latter may be

disregarded, and attention given wholly to the breeding places of those species responsible for the transmission of malarial fever.

With regard to man as a host, it is necessary to have some knowledge of the limits of his infectiousness, i.e., the number of sexual forms of the malarial parasite necessary to infect susceptible mosquitos.

Besides the question of hospitable species of Anophelines, there are other matters of much importance, such as latent malaria; the effect of quinine on the parasites in man; the value of various larvacides; algacides; agents destructive to ditch grass, and a knowledge of the quality of wire screening, and the size of the mesh necessary to keep out mosquitos.

OUTLINE OF THE SUBJECTS CONSIDERED:

Anophelines of this region.
Collection of larvae.
Breeding out mosquitos and methods of feeding.
Biting—infecting experiments.
Estimation of gametes.
Care of mosquitos after feeding.
Method of examining for zygotes and sporozoites.
Description of the malarial parasite in the mosquito.
Table of infecting experiments.
Notes and conclusions from table of infecting experiments.
Limit of infectiousness of man.
Notes on the bionomics of Anophelines.
Effect of salt or sea water on larvae.
Experiments with larvacides.
Experiments with agents destructive to vegetation, grass and algae.
Experiments with screening of various mesh.
Relative value of wire screening of various composition, based on practical tests and chemical analyses.
Note on the value of the practice of killing Anophelines found in quarters and barracks.
Effect of quinine upon the parasite in mosquito and man.

The following is a list of Anophelines of the Canal Zone:—

Cellia argyrotarsis, R.D.
Cellia tarsimaculata, Goeldi
Cellia(?) gorgasi, D.K.
Cellia albimana, Wied.
Anopheles (?) cruzii, D.K.
Anopheles (?) apicimacula, D.K.
Anopheles (?) punctimaculata, D.K.
Arribalzagia (?) malefactor, D.K.
Anopheles (?) eiseni, Coquill
Anopheles francisanus, McCrack
Anopheles pseudopunctipennis, Theob.

The above eleven species of Anophelines have been collected in the Canal Zone during the past five years. They are not taken, nor do they exist in their breeding places, in anything like equal numbers. For example: Only one specimen of *Ce. (?) gorgasi* has been found. Of the eleven species, the commonest ones are *Ce. albimana*, *A. pseudopunctipennis* and *Arr. (?) malefactor*, but this again must be qualified by stating that the predominance of a species varies from season to season and from place to place. In certain villages, upon going through the barracks only *Ce. albimana* will be found, while in other villages, from five to ten per cent. of the mosquitos will be *A. pseudopunctipennis*, and at Ancon during October, 1908, 27 per cent. were *A. malefactor* and 72 per cent. *Ce. albimana*. Mr. A. Busck, of the Bureau of Entomology, United States Department of Agriculture, who collected and made observations on Zone mosquitos during 1907, gave it as his opinion that *A. pseudopunctipennis* was the commonest Anopheline during the period of his stay.

The necessities of the canal operations in excavating and filling, change the topography of districts and localities so as sometimes to convert salt marshes into fresh water ponds, or to make tracts of land containing few Anophelines, into a vast swamp in which they luxuriate. On the other hand, swamps and breeding places may be drained or filled in the work of excavation. These factors, among others, influence the number and variety of species in a locality.

The commoner Anophelines of the Canal Zone may be divided into three groups:—

(A) The white hind-footed group comprising:
Ce. argyrotarsis,
Ce. albimana,
Ce. tarsimaculata.*

(B) The leg uniformly coloured group comprising:
A. pseudopunctipennis,
A. franciscanus.

(C) The spotted leg group comprising:
Arr. (?) malefactor,
A. (?) apicimacula.

These groups present well-marked differences in the markings of adults, in the breeding habits and anatomical characters of the larvae, and, as will be shown, they possess varying susceptibilities to malaria.

The following are descriptions of the species of Anophelines of the Canal Zone:—

Cellia argyrotarsis

Thorax with mesonotum bluish-grey, with three more or less longitudinal lines and with pale scales over the mesonotum, and sometimes traces of two dark lateral spots. The abodmen dark, dusky-brown, with a few creamy scales. Legs covered with dark scales, with some of the tarsi apically white banded; last three joints of hind legs pure white, and also apex of first; costa dark with two distinct and several smaller pale spots.

♀ Head black, with white upright spatulate scales in front, black behind and at the sides, a tuft of white hairs projecting forwards between the eyes. Eyes black; antennae dark, with pale silky pubescence and brown hair; basal joint dark, a few patches of white scales on the first few basal joints; palpi covered with long black scales, especially towards the base; apex pure white, and there are also two narrow white rings on the apical ends of the joints; ventrally, the penultimate joint has a number of yellowish-white scales, which sometimes seem to form almost a ring; proboscis clothed with short dark scales.

Thorax with a bluish-grey sheen, with three more or less distinct longitudinal lines, the middle one most distinct, and of a purplish hue, with pale scales scattered over the mesonotum; scutellum dark towards the middle; mesonotum deep brown; pleurae dark, with here and there frosty tomentum (there are traces of two dark lateral spots on the mesonotum, which are clearly seen in the St. Lucia specimen).

Abdomen dusky purplish-brown, clothed with creamy yellow scales, especially in the middle region of the segments; the segments have lateral tufts of grey scales on the posterior borders, projecting from the sides; hairs long, deep bright brown; viewed with a pocket-lens the abdomen is almost black in ground colour; in other specimens dull yellowish reflections may be seen.

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* According to Theobald (Monograph of Culicidae, Vol. V, p. 69) identical with *albimana*.—Ed.

Legs yellowish, covered with dark brown scales; first two tarsi of the forelegs apically white, last two joints dark brown, four midtarsi also with small pale apical bands; mid metatarsi and first two tarsal joints with minute apical yellow bands, last two indistinctly banded; in the hind legs the last three joints are pure snow white, and also the apex of the first; ungues very dark.

Wings with the costa dark, with four distinct and several smaller white patches; there are also numerous patches of dark scales, which vary to some extent, over the wing areas; in the ♀, from which this description is taken, the fourth long vein is covered with pale dusky scales, whilst in a ♀ from St. Lucia, it is creamy white; halteres with pale stem and fuscous knob. Length, 4 to 5 mm.

♂ Palpi dark brown, with scattered white scales, especially on the last swollen joints; hair-tuft pale; there is a pale ring at apex of the apical and base of the penultimate joint; antennae brown, with brown plumes; proboscis brown and narrow. The white scales on the head extend nearly over the neck; scales on the thorax white; the larger ungues of the fore-feet biserrated. Length, 4 to 5 mm.

During the period in which these experiments were being conducted I received very few specimens of this species, the sources being Miraflores, Ancon, Culebra, Paraiso and Corozal. Two specimens of *Ce. argyrotarsis* bit a patient having one crescent to 200 leucocytes and neither became infected. The patient was possibly an unfavourable case, and the experiment was not controlled by biting susceptible *Ce. albimana* at the same time. On December 2nd, from some Anophelines collected in labour cars at Corozal, one specimen of *Ce. argyrotarsis* was found containing a malarial zygote, 29μ in diameter, with fine discrete pigment.

Cellia tarsimaculata

This mosquito resembles *Ce. albimana* very closely, except for the different arrangement of the white bands on the palpi. This mosquito was found to transmit malaria.

Cellia albimana

This form resembles the type in all respects except that the last tarsal joint in the hind-legs has a very distinct and persistent deep black basal band. The thorax is rather browner in some specimens, and there are only two white bands to the ♀ palpi. The forelegs have dark-scaled femora, pale underneath, with a small white knee spot, the tibiae dusky-scaled and also the metatarsus above, pale below, apex white; the first two tarsi have yellow apical bands, the third dark, and the last clay coloured; mid legs with a large white spot near the apex of the femora; mid tarsi not definitely banded, but with a faint pale band sometimes at the apex of the metatarsus; the hind legs are dark brown, with the second, third, and apex of the first tarsal joints pure white, the last joint white, with a distinct black basal band; ungues as in the type. Wings much as in the type, but the pale scales are more yellow in colour. Length, ♂ 3·5 to 4·5 mm.; ♀ 4 to 4·5 mm.

This was the commonest species of Anopheline received as adults or larvae during the period embraced by this work, and was found to transmit both malignant tertian and simple tertian malaria.

Cellia (?) gorgasi

Palpi as long as the proboscis, mostly black scaled, the terminal and penultimate joints light scaled, except at the bases and apices; mesothorax grey with fine brown scales, a black spot in front of the scutellum, a pair of sublateral black spots medially; wings with the veins scaled in black and white, two very

large black patches on the costa and a smaller one towards the base and a smaller one at the apex as in *Ce. albimana,* Weid. The rest of the wing is too much denuded to describe. Abdomen with groups of outstanding scales laterally at the apices of the segments, the dorsum clothed with yellow scales on a dark brown ground, the lateral tufts black. Legs mostly black-scaled, hind legs with the apical half of the second, the third, and the base of the fourth joints white-scaled, the remainder of the fourth and basal half of the fifth segments black, the third joint with a large black patch on the under side which reaches from near the base to beyond the middle. Length, 3·5 mm.

A single adult female of this species was collected by Mr. A. H. Jennings.

Anopheles pseudopunctipennis

Wings much as in *A. punctipennis,* Say, but the fringe with yellow spots. Legs, long, unbanded, brown, pale at the base. Fore ungues of ♂ unequal, mid and hind equal and simple.

♀ Antennae brown, basal joint testaceous, base of the second joint pale, and also a small pale band at the base of all the following joints: proboscis dark brown; labella yellowish; palpi dark brown, densely scaled at base; apex yellow, and also two narrow yellow bands below, slightly hairy, hairs black, except at the apex where they are yellow; clypeus dark brown. Thorax yellowish-brown (denuded), with a dark patch on each side of the mesonotum behind; metanotum deep brown; pleurae yellowish brown, with darker brown patches. Abdomen brown, the segment paler at the base, hairy. Legs deep brown; coxae, trochanters and base of femora pallid; knee spot pale; ungues equal and simple. Halteres with pale stem and fuscous knob.

Wings with two yellowish white spots on the upper costal border, rest of the edge black, rather densely scaled; first submarginal longer and narrower than the second posterior cell, its stem nearly as long as the cell; mid cross-vein a little nearer the base of the wing than the supernumerary cross-vein; posterior cross-vein still nearer the base of the wing; scales of the wings disposed as follows:—First long vein with three distinct large white spots, one at the base, one underneath a large costal spot, and one between; second long vein with a dark patch near its base, all the lower branch of the fork-cell dark, and most of the upper; third long vein mostly yellowish-white, with two black patches, one towards the base, and the other towards the tip; fourth long vein mostly pale, with two small black patches, branches of the fork-cell all dark scaled; fifth long vein with a black spot near the base, rest mostly yellow, upper branch of the fork mostly dark, a small yellow spot at the apex and another towards its base, lower branch mostly yellowish, with a black apical spot; sixth vein with the basal half creamy, the apical half dark, except a small yellow patch where it joins the wing border; fringe brown, with yellow spot at the junction of each vein. Length, 5 mm.

♂ Last two joints of the palpi swollen and clavate, pale; basal joints dark brown, densely scaled with deep brown scales, with a narrow pale band not quite as long as the thin proboscis, which is brown with yellow labella; antennae grey, with narrow brown bands and flaxen brown hairs, the apical joint about half the length of the penultimate joint; basal lobe of the genitalia simple, claspers long and thin; fore ungues unequal, the larger one uniserrated, the smaller minute and simple; mid and hind ungues small, equal and simple. Wings much as in the ♀ but the fork-cells shorter. Length, 5 mm., with proboscis 7·5 mm. Habitat, Grenada (Dr. Hayton, per Dr. Daniel). Time of capture, February.

Observations—Very like *A. punctipennis,* Say, but can at once be told by the wing fringe being spotted at the apex of each nerve, and by the marking of the sixth long vein. The description is drawn up from two specimens in balsam, so

that the scale structure is not evident. It is so very distinct, however, that it can easily be identified by the characters given below. I succeeded in infecting four specimens of this species.

Mr. August Busck found this species to be the commonest and most widely distributed one in the Zone during the season in which his collections were made, April-July, 1907.

Anopheles franciscanus

Male: Head dark brown, with short, dark, erect scales towards nape, emarginate and slightly forked, vertex and anterior part of occiput, with short, light brown scales not forked, a tuft of light brown hairs projecting forwards between the eyes, a row of similar hairs projecting forwards encircling the eyes posteriorly; eyes deep purplish brown; antennae about two-thirds length of palpi, yellowish-brown hairs, basal joint dark brown; palpi equalling proboscis in length with emarginate scales from base to tip on under and outer surfaces, those upon outer surface dark, upon under surface light, long light hairs covering distal third, becoming short and stout at the apex, a slightly banded appearance at base of three distal segments; two distal joints spathulate, proboscis scaled except labella, labella covered with medium stout setae, a few light hairs at apex.

Thorax: Prothoracic lobes dark; mesothorax dark brown at the sides, with scattered light hairs, a broad light-brown patch in the middle; within this light area a median line and obscure lateral lines; scutellum light with single horizontal row of hairs; metanotum dark without hair; halteres dark, covered with thick pubescence and emarginate scales; stalks light without scales.

Abdomen, basal area of each segment light, covered sparingly with long, light hairs; two stiff hairs on posterior margin of distal segment, stout hairs on margin of genital lobes.

Legs, coxae and trochanter light; trochanters, femora, tibiae, and tarsi covered with short, dark, emarginate scales and setae; ungues of front legs very unequal, the larger one with a large median tooth and a smaller basal lobe; middle ungues covered, with blunt basal lobes; posterior ungues equal, simple; posterior metatarsus slightly longer than tibia.

Wings with dark costa, with two distinct, nearly equal, yellow spots—one at distal end of subcostal vein, one at and involving distal end of first long vein; fringe dark, with a yellow spot at the end of each vein except at the end of the sixth; the first spot carried on to the first long vein, the apical spot carried past long vein on to the upper branch of the second long vein; the second long vein dark except for a few basal light scales; third long vein yellow in the middle, dark at the base and apex; light area at base of third long vein, carried over the fourth on to the upper branch of the fifth, with a few light scales at base; main branch of fifth long vein light, except at base and apex; distal half of sixth long vein dark, except at apex, basal half light; sub-costal with a light spot carried to the first long vein (in one specimen the light spot on sub-costal missing); third long vein prolonged slightly into the basal cell; first sub-marginal longer and slightly narrower than second posterior cell, stem twice the length of the cell; stem of second posterior cell prolonged to base of wing; supernumerary cross-vein adjacent to, or but very shortly removed from mid cross-vein and equal to it in length when removed nearer to apex of wing; posterior cross-vein a little longer than mid cross-vein and varying in distance from it, from one-half to almost twice its own length; third long vein prolonged slightly into the basal cell, darkest scales on costal, sub-costal and first long veins.

Palpi of the female equalling proboscis in length, light area at base of three distal segments, giving a banded appearance, clothed with scales, short hairs and

setae, as in male, distal joints not spathulate; legs with the ungues equal, otherwise with the male.

No specimens of this species were infected, but as they are so close to *A. pseudopunctipennis* it might have been possible to infect a few if a large number had been used.

Arribalzagia (?) malefactor

♀ Palpi long, clothed with brown scales and black outstanding ones, which are grouped more or less in tufts, heaviest on the basal portion, a slight sprinkling of lighter scales among the brown ones, particularly at the bases of the dark tufts; occiput black scaled, the eyes margined with white above, and where they join it a tuft of white hairs; mesonotum grey with reddish and bluish tinge and small dark freckles tending to form longitudinal rows, sparsely distributed narrow yellowish scales, a spot at the base extending over the middle of the scutellum and two small sub-lateral back spots medially, all three of these show a lighter margin; abdomen slender, grey, with lateral tufts of outstanding black scales at the apices of the segments; legs with the femora and tibiae black, freckled with white; on the hind tibiae yellow scales predominate; tarsi black, ringed with yellowish-white; on the hind legs the first tarsal joint is dark at the base, light at the apex and has six white rings of different lengths, second joint narrowly white at base, broadly so at apex, with a moderately broad white ring near the middle and another narrower one between it and the base, third and fourth joints white ringed at base and apex with a broad central white ring, apical segment entirely whitish scaled; wing spotted, black and white, a large black patch margined with white on the costa near the middle, more basally a smaller costal patch and towards the apex another large one, all margined with white, scaling of the veins in patches of black and white scales, the third vein with a small black spot at the base, the sixth vein with many black dots and dashes. Length, 4·5 mm.

♂ Palpi with the apical portion clubbed, clothed with yellow scales with golden lustre, a narrow dark ring at the middle of the club, the shaft ringed with dull ochreous at the apex and at the constriction and broadly marked with the same colour on the apical portion; antennae pale brown and ferruginous with silky lustre. Length, 4·5 mm.

This large and beautiful Anopheline was received in good numbers from Miraflores and Ancon. Its name, however, appears to be a misnomer, for it could not be infected with malaria under the most favourable conditions.

Anopheles (?) apicimacula

As in *A. strigimacula*, D. and K., but with a distinct black costal apical spot on wing.

Anopheles (?) strigimacula

Proboscis black; palpi as long as the proboscis, black, a few whitish scales at the bases of the last two and middle of the long joint. Occiput black, clothed with erect black scales, a group of white ones in the centre of the vertex, a tuft of pale hairs at the vertex.

Mesonotum narrow, elongate, greyish, pruinose, a black spot below the lateral angle and one on the ant-scutellar space; vestiture of fine pale hairs arising from small black tubercles. Scutellum collar-like, greyish, with a black spot in the middle, clothed with pale bristles. Pleurae and coxae blackish with fine hairs, the coxae with patches of white scales.

Abdomen with the tip truncate, brownish black, clothed with numerous fine pale hairs; a row of lateral segmental posterior tufts of black spatulate outstanding scales; beneath with tufts of black scales and with scattered white ones.

Wings hyaline, the petiole of the second marginal cell as long as its cell; basal cross-vein distant about its own length from the anterior cross-vein; scales of the veins ovate, white on the costa and first vein, pale yellow on the others, with black scales and spots as follows :—

Three costal spots, the first small, involving two veins, the others large, involving three veins, the membrane beneath infuscated; no apical spot; costa and first vein with two or three little black spots between each of the large ones, the outer spot involving the base of the fork of the second vein; each fork with two little spots beyond; third vein with two spots at the base and two at the tip; fourth vein with a spot at the base, a large one involving the base of the fork, three on the upper branch and two on the lower; fifth vein with some black scales at the base, five spots on the upper fork, two on the lower; sixth vein with some irregular black scales toward the base, a spot in the middle and one at the tip.

Legs long and slender, black, speckled with white. Femora with about eight spots; tibiae with about fourteen, being about as many black scales as white ones; hind tarsi with ten spots on the first joint, second, third and fourth joints white at the base and tip, with a ring beyond the middle; fifth joint all white. Front tarsi with narrow white rings at bases and apices of the joints, the last entirely pale; mid tarsi not distinctly ringed. Claw simple. Length about 5 mm.; of the wing, 4 mm.

Anopheles (?) punctimaculata

As in *A. strigimacula,* D. and K., but the last vein with a row of black dots.

Only one specimen of this species is known. It was taken at Colon (W. M. Black, collector).

Anopheles (?) cruzii

This species was examined only in the larval state, through the kindness of Mr. A. H. Jennings. No adult specimens were obtained and none ever received from or taken from quarters. This is significant on account of the peculiar tree-living habits of this species, and the probably groundless fear that it might be a malaria carrier.

Anopheles (?) eiseni

Near *A. maculipennis,* but with a patch of whitish scales on the first vein before its middle and another at its apex, also the apical fourth of the hind tibiae is yellowish-white. Halteres black, the stem whitish; coxae and a vitta on lower part of pleura, yellow, femora yellowish-brown, apical fourth of hind tibiae yellowish-white; antennae of male whitish, the first joint, last two, and fascia on each of the others, brown; scales of palpi black, those of apex and two bands in the female, three in the male, white; scales of occiput black, those in the middle of upper part white; mesonotum greyish pruinose, marked towards each side with a velvet black vitta; scales of abdomen black, the hairs yellowish, scales of femora and tibiae mixed black and whitish, those on the apical whitish portion of hind tibiae white, those on the tarsi black; tarsal claws on female simple; wings hyaline, the veins and scales brown, a dense patch of black ones at base of second vein, a larger one on the cross-veins and a smaller one at bases of first sub-marginal and

of second posterior cell, a small patch of yellowish-white scales on first vein before its middle and another at its apex, the latter spot encroaching upon the costal vein. Length, 3·5 mm.

It would have been of considerable interest to determine the susceptibility of *A eiseni* and *A. cruzii* to malaria on account of their peculiar tree-living habits, but it was almost impossible to obtain larvae of those species; and among hundreds of Anophelines caught within quarters and barracks which were examined and dissected, no specimens of this species were ever seen. It is extremely unlikely that they play any part whatever in the transmission of malarial fever in the Canal Zone at this time. The larvae of these species proved extremely interesting in comparison with the commoner species, such as *Ce. albimana*, *A. pseudopunctipennis* and *Arr. (?) malefactor*, the anatomical characters of the former indicating definitely a very marked alteration in habits.

COLLECTION OF LARVAE

In order that a large number of adults could be kept on hand from day to day, it was arranged that sanitary inspectors at the various districts along the Panama Railroad should send bottles containing larvae and pupae to the laboratory daily. Special collections of larvae were also made and excursions to breeding places made from time to time. Upon receiving them at the laboratory, larvae were transferred to a glass moist jar partly filled with fresh water. Predaceous larvae, such as dragon-fly larvae were removed or killed and the Anopheline larvae transferred to feeding tanks containing algae and organic débris. These glass breeding tanks were placed on a table in front of a window having an eastern exposure so that they got direct sunlight for a few hours in the morning. The water in the breeding tanks was kept fresh and free from fouling by passing a jet of air through it with a Pacquelin cautery bulb, having a heavy glass perforated tip. This proved to be a very important addition to the technique of breeding out larvae. For shade and shelter a few *Lemma* plants were placed in the tank.

Several writers, collectors, and malarial investigators have mentioned the difficulties attendant upon the breeding out of Anopheles mosquitos from ova or very young larvae. Others have not mentioned the difficulties encountered or have not described the means used to obviate them. Banks, in the *Philippine Journal of Science*, Vol. II, No. 6, December, 1907, states, 'In laboratory 'breeding experiments the plants in the water begin to die within

'three to five days, while the larvae appeared to feed in a desultory 'manner. The time for their natural transformation to the pupae 'comes and goes and they still remain as larvae. The foulness of 'the water, due to organic decomposition, appears not to affect 'them, but on the other hand the lack of proper food seems to cause 'them to remain in an indefinite larval state until after three weeks 'or more they gradually begin to die.'

A very simple and satisfactory method for keeping the breeding tanks fresh and clean and free from decomposition was devised. The principle involved is that of aeration—preventing the development of an anaerobic condition in the water of the breeding tank by passing a fine jet of air through the water of the tank once or twice a day. It is well known, of course, that if natural water from streams, pools or ditches be placed in a glass tank, exposed to diffuse sunlight and undisturbed, after a few days the chlorophyll-bearing plants begin to disappear, a pellicle forms on the surface of the water, which contains bacteria, spirilla, flagellated protozoa, amoebae, etc. Beneath this surface film decomposition goes on rapidly, chlorophyll-bearing forms are destroyed or become encysted, and the various insect larvae and water-bugs die. The bacteria in the deeper portions of the water are largely anaerobes, and are associated with the putrefactive decomposition of the vegetable matter and animal matter in the tank. Banks describes exactly the effect on mosquito larvae of this putrefying vegetable matter. The odour arising from the tanks suggested the necessity of aeration to prevent the growth of the anaerobic bacteria. Acting on this suggestion an air jet was devised by attaching a thick glass rod, having a fine capillary central canal to the double bulb of the Pacquelin cautery apparatus and the breeding-out jars aerated for a minute or two morning and evening. The results were highly satisfactory, for the tanks were by this means kept clean and wholesome. The algae remained green and vigorous, the larvae active and developed rapidly into pupae, the water remained quite clear, in fact, with its floor of sand and clay and a few sprigs of green aquatic plant, such as *Lemna*, looked as tempting to drink as spring water. The temperature of the water in the tanks ranged between 72° and 84°.

BREEDING OUT—METHODS OF FEEDING

Pupae were collected in the morning and evening and placed in breeding-out tubes half filled with water and plugged with cotton. Each morning the newly emerged mosquitos were transferred to biting-jars. These biting-jars were modified from those described by Stephens and Christophers. After trying pickle jars and malted milk jars with much impatience, jars made of lantern chimneys were used. These were covered on both ends with crinoline gauze, fastened with a string or a strong rubber band. Inside the jar was placed a circular ring platform of stiff paper, which many of the mosquitos used as a resting place. About twenty mosquitos would be placed in a jar over a small Stender dish* containing water on a Petri dish cover with a raisin or a piece of date for food. The jar would then be placed on a shelf in a dimly-lighted place, protected from ants by kerosene cups. Adult mosquitos may be kept alive for several days in such jars if they are fed with dates or raisins and a few drops of water, but if fed daily they will not bite and suck blood with alacrity. Anophelines do much better if they have one or two preliminary blood-meals before being fed with dates, raisins or bananas. In several experiments it appeared that in the mid-gut of mosquitos gorged with stale banana and the associated bacteria and yeasts, the fermentative acid contents either destroyed the flagella of gametes, or in some other way prevented the development of zygotes in the mid-gut of susceptible mosquitos. In one experiment a patient whose blood contained almost as many crescents as leucocytes was bitten by four varieties of mosquitos; three of the four being susceptible species, yet none became infected. This failure might be attributed to the feeding on bananas.

Until the mosquitos were used for biting they were fed with dates, raisins and bananas, whenever they were to be kept for biting and dissections, it was found that those fed on dates and raisins gave much better dissections, while the mid-guts of banana-fed mosquitos frequently contained many yeasts and bacilli which caused the death of some of the mosquitos, apparently as a result of fermentation, and frequently yielded partly softened, tender and

* A ' Stender-dish ' is one with a grooved lid fitting the rim of the dish.—EDS.

disintegrated intestinal tracts, which were removed intact with difficulty, and were occasionally lost. Bananas should never be used as food when dates or raisins can be obtained.

Mosquitos were kept in these biting-jars until a suitable case of malaria presented. The blood of patients selected to be bitten contained gametes or sexual forms of the malarial parasite in their circulating blood in numbers sufficient to infect susceptible anophelines. The method of determining this point will be considered later.

BITING AND INFECTING EXPERIMENTS

The earlier biting experiments were conducted at about 8 o'clock in the evening. After selecting a patient the jar of mosquitos was placed on the patient's forearm and covered with a heavy towel to prevent the disconcerting effect of light. Females several days old, that had been fed exclusively on dates or raisins, would generally bite greedily, and would feed on successive or alternate nights if given an opportunity. Most females would bite twenty-four or more hours after emerging, but before that period they would generally make no attempt to do so. When a jar is placed over a patient's forearm the mosquitos that are going to bite will almost always do so within a few seconds. If they show no inclination to visit the arm, a few gentle puffs into the opposite end of the jar, through the crinolin gauze, will make them change their position and frequently take up one on the patient's forearm. Another method that was used successfully was to have the patient's bed in the dark. If the jar were then gently tapped two or three times and aimed at a light down the ward some distance away the mosquitos would always take up a position on the gauze facing the light. The patient's forearm could then be carefully interposed and placed on the gauze, when the mosquitos would frequently take the hint and feed. Strong lights are powerfully attractive to female Anophelines and interfere considerably with successful bitings unless the jars are darkened by means of a thick cloth, the patients taken into a dark room, or the mosquitos very hungry.

Later, it was more convenient to conduct the biting about

4 o'clock in the afternoon, and on these occasions it was generally necessary to cover the jars well with a thick towel.

Two classes of patients were used: Spanish labourers and West Indian negroes, the former rarely objected to being bitten, the latter occasionally fretted a little. Both classes of patients were rather obtuse mentally and occasionally would not feel the bite, or would not be able to tell correctly the number of them. One reason for this is to be found in the limits of the number of separate points of pain appreciable within a radius of three inches—the biting area of the jar.

The Spanish patients' hands occasionally reeked with the odour of cigarette smoke, and a few of the West Indians had used citronella to prevent sand-flies from biting, but in neither instance did this interfere with the feeding of mosquitos. The hungry females, if undisturbed, will gorge themselves with blood and not infrequently will expel a drop or two of bloody fluid per anum. This not only occurs at the first feeding but has been observed at subsequent feedings.

After as many of the females as will bite have done so, the jars are taken to the laboratory, one of the gauze covers carefully removed and at the same time replaced by a card which is slipped out when the jar is placed on a Petri dish. The Petri dish contains a small Stender dish with a few drops of water and a split raisin or two. The jar is then placed on an ant-proof shelf in a dimly-lighted place, and under these conditions, with fresh food and water, Anophelines may be kept alive for two or three weeks. If one has fed mosquitos late in the evening and does not care to transfer or uncover them by artificial light, the gauze may be moistened with a few drops of tap water and the jars placed on the shelf until morning.

At the time of biting the patient, two or three good blood films for staining were taken and differential counts of leucocytes as well as the proportion of gametes to leucocytes made the following morning. By this means, and by an occasional leucocyte count, it was possible to estimate from films the number of gametes ingested by the mosquito in feeding.

ESTIMATION OF GAMETES

The estimation of the number of gametes ingested by the mosquito gives one an idea as to the grades of infection to be encountered in the mosquito, and this has indirectly a practical bearing which will be discussed later.

Leucocyte counts taken during convalescence in malarial fever generally show a number not far removed from the normal. It was possible, then, to estimate the number of gametes by counting the number met with while enumerating the leucocytes during the differential count. Such a gamete count was made from the blood of every patient bitten.

Mosquitos were weighed before biting and after biting, and the amount of blood ingested estimated in this way.

Weights of Mosquitos

	Weight.
Ce. albimana bred in laboratory, twenty-four hours old, mid-gut empty	0·0008
Ce. albimana bred in laboratory, moderate blood feeding	0·0016
Ce. albimana caught in labour-cars, some blood in mid-gut, half developed ova	0·0019
Ce. albimana caught in labour-cars, much blood and early development of ova	0·0035
Ce. albimana caught at barracks, blood in mid-gut, no development of ova	0·0018
Ce. albimana caught at barracks, blood in mid-gut, no development of ova	0·0021

The average weight of mosquitos twenty-four hours old was 0·0008 gramme, while the average weight of mosquitos from the same brood that had bitten and taken a moderate amount of blood was 0·0016 gramme. The average weight of blood ingested being then 0·0008 gramme, approximately 0·001 gramme. Assuming the specific gravity of blood in malarial fever with slight anaemia to be 1·050, then 105 : 100 : : 0·0008 : 0·000761 = the volume of 0·0008 gramme of blood. Now if there were 22 gametes per 100 leucocytes, as in Experiment 38, and 6,500 leucocytes per mm.^{3} as they were, by actual count, there would be $22 \times 65 \times 0{\cdot}761 = 1088$ gametes ingested. If, under the most favourable circumstances, there are an equal number of male and female gametes, there should have been about 1,632 zygotes in this mosquito's mid-gut after three feedings,

but as a matter of fact, there were only about fifty, showing a loss of about 97 per cent. This loss may be partly explained by an observation on the fate of gametes *in vitro* and *in vivo*, when it was noticed that fully 50 per cent. of gametes were phagocyted by polymorphonuclear leucocytes. This last is truly surprising, because this type of leucocyte in the circulating blood of man rarely plays any part in malarial phagocytosis, save in pernicious infections, when it will then engulf parasites and pigment. Usually it is the large mononuclear and endothelial cells which phagocyte malarial parasites and pigment.

In Experiment 41, blood specimens from a patient taken on admission contained 92 crescents per 100 leucocytes. Fresh preparations examined fifteen to twenty minutes after being taken, contained many pigmented extra-cellular parasites, some with quiescent pigment, others with pigment dancing in a circle around a granular centre. There were free flagella in some fields—two in one field. One flagellum was seen attached to several red blood cells and was moving with extreme violence, but without becoming detached. Quite a number of the gametes became phagocyted by polymorphonuclear leucocytes, one of the latter had phagocyted two gametes. Stained specimens showed numerous gametes within the polymorphonuclear leucocytes; one free, detached flagellum was seen. Some of the gametes were globular with linear pigment and a large chromatin dot, while others contained granular pigment, surrounding a chromatin ring, staining interruptedly and having an achromatic space on its interior and exterior.

CARE OF MOSQUITOS AFTER BITING

The mosquitos may be fed nightly or every other night from patients, or, if it is desired to ascertain the rate of development of zygotes, they are fed on dates after a single biting. The mosquitos should be kept in the biting jars with fresh food and very little water, just enough to favour oviposition and to keep the air within the jar moist, but not so much that they would be drowned.

It is very necessary that the infected mosquitos be protected from ants; otherwise valuable specimens that die during the night will be removed, with nothing but wings and legs to mark the loss.

Mosquitos may be transferred one at a time from the biting jar, and from day to day killed and dissected. Chloroform and cyanide may be used for killing. Chloroform is more conveniently used, but cyanide yields better preparations when it is desired to preserve most of the mosquito for identification, for the reason that cyanide causes the mosquitos to spread their wings.

METHOD OF EXAMINING FOR SPOROZOITES AND ZYGOTES

First it should be said that successful dissections can only be obtained with killed specimens. Mosquitos that have been dead twelve or more hours, particularly if they had fallen on the water, have become so macerated that the cells of the mid-gut or salivary glands separate and float away, it being impossible to retain the organ intact. When an examination of the salivary glands is desired, the wings and legs of the mosquito are trimmed off with a small, sharp entomological knife. The distal half of the thorax, with the abdomen, is removed by a transverse, clean cut, the mosquito being laid on a piece of white cardboard. With a small pair of forceps the proboscis is grasped, and the specimen laid on a drop of saline solution on a Stender dish cover under the dissecting microscope. The chitinous covering of the thorax, just behind the nape, is carefully slit, or torn, and the muscle organ beneath loosened slightly; then by pulling out the proboscis with one needle and holding the chitinous thoracic covering with the other, the salivary glands will be drawn out. They should be cut off from the head by a small, very sharp knife, and picked up with the point of a bright needle and placed in saline solution, 10 per cent. formalin, or films made for staining.

The sporozoites will be seen either free or in epithelial cells, or in the duct of the salivary glands; appearing as thin, slightly curved, spindle-shaped bodies, placed side by side, frequently as though matted together.

METHOD OF EXAMINING FOR ZYGOTES

The identified mosquito is laid on a piece of white cardboard, the abdomen cleanly and carefully removed by a transverse cut, just behind the thorax, and placed on a glass slide, or better, the under

surface of a Stender dish cover, with a drop of saline solution. Under the dissecting microscope with the reflector properly adjusted, the hind-gut, mid-gut, Malpighian tubules and ovaries are withdrawn by pulling carefully on the last abdominal segment with one needle and holding the first abdominal segment by a corner with another needle. The mid-gut is separated from the hind-gut and Malpighian tubules as well as possible and transferred to a slide on the point of a needle, which should be sharp and well burnished, where it may be examined in saline solution, formalin, or by other methods. When the mosquito has been dead several hours, the mid-gut cannot be withdrawn intact. In this event, it is generally necessary to split the chitinous abdomen open, and search carefully for as much of the mid-gut as may have held together. Zygotes can be detected with a low-power lens, Zeiss, 16 mm. objective and 8 and 12 oculars. If an absolute identification cannot be made at the time, all parts excepting the abdomen must be preserved intact, mounted with experiment number attached for final identification.

DESCRIPTION OF THE MALARIAL PARASITE IN THE MOSQUITO

As stated above, it was found that many gametes were phagocyted in the mosquitos' stomach (50 per cent. or more) by polymorphonuclear leucocytes. This materially diminishes the number of fertilised ookinets which reach and enter the wall of the mosquito's mid-gut. Notes from the following experiment throw some light on the changes occurring in crescents *in vitro*. The phagocytosis of crescents in the mid-gut was demonstrated separately, but films do not permit as careful study as those *in vitro*.

EXPERIMENT NO. 20.—Blood contains, December 30, 67 crescents per 100 leucocytes. Temperature 97° continuously.

DIFFERENTIAL COUNT OF LEUCOCYTES

Polynuclear	68	
Large mononuclear	4	
Small mononuclear	20	67 crescents per 100 leucocytes.
Eosinophil	7	
Mast	1	

In the fresh blood film, when it is drawn, numerous crescents are seen, but within five to ten minutes half the number of crescents have become vesicular and their pigment dancing; several have thrown out flagella, which are still attached after fifteen to twenty minutes. These vesicular gametes have become phagocyted by polymorphonuclear leucocytes; some of them at this period have parted with their flagella, because several free flagella were detected in the film. Stained preparations fixed immediately upon drawing the blood show nothing but crescentic forms, but films that have been kept moist from five to ten minutes before staining show that a very marked change has taken place in the crescents, many of them becoming either globular or irregular and distorted. One film fixed after ten minutes, and stained, showed nine crescentic forms, eight globular forms, with discrete pigment and several chromatin dots, some of which are certainly microgametes, and eleven irregular forms, as though becoming globular, or as crescents losing their stiff outline, and becoming flexible.

After fertilisation the microgametocyte becomes elongated (wandering vermicule, ookinet). Illustrations of wandering vermicules (after Grassi) are very much like the eleven irregular forms seen in the blood just described. These, then, may represent fertilised gametes.

The fertilised gamete, or ookinet, if it be not phagocyted, has abundant time to wander out of the blood clot and reach the gut wall, for the blood-meal of the mosquito is usually not expelled until after twenty-four hours.

The earliest form of the malignant tertian zygote was detected in the wall of the gut after the expulsion of the blood-meal, or after two and a half days. Satisfactory dissections and examinations cannot be made until the blood-meal has been expelled; consequently, after several trials, sixty hours after a feeding was the earliest period at which a search was made for zygotes.

In Experiment No. 204, a specimen of *Ce.* (?) *tarsimaculata* was killed sixty-five hours after a single feeding from a patient whose blood contained ten crescents per 100 leucocytes. Upon examination there were about fifty zygotes in the mid-gut. They were slightly oval in outline, with closely clumped quiescent pigment, and very little cytoplasm showing beyond the pigment, the diameter being

about 5 μ. The zygotes become larger each day, though they do not always appear to grow at equal pace.

This variation in size may depend on location in the gut wall and its relation to nutrition. On the whole, however, the gradual increase in the size of the zygote is fairly uniform, as the following table shows: —

Experiment No.	Age of Zygote	Type of Parasite.	Size of Zygote in μ
16	4–4½ days	Simple tertian	12 × 16·5
16	4–4½ ,,	,, ,,	17 × 20
32	5 ,,	,, ,,	19·5
16	8½–9 ,,	,, ,,	48, 54
16	8½–9 ,,	,, ,,	48 × 54, 55·5 × 66 (Sporoblasts formed)
18	Sporozoites in	salivary glands. Smple tertian	
204	65 hours	Malignant tertian	5
9	60 ,,	,, ,,	9 × 11·25
41	2½ days	,, ,,	9 × 10·5
38	3 ,,	,, ,,	7 × 10
43	3 ,,	,, ,,	7 × 10
41	3½ ,,	,, ,,	12 × 15
38	4 ,,	,, ,,	12 × 15
33	3¾–4¾ ,,	,, ,,	12 × 13·5
42	4¾ ,,	,, ,,	21
36	5 ,,	,, ,,	21
11	6½ ,,	,, ,,	22, 28
38	7 ,,	,, ,,	45 × 57 (Contains sporoblasts)
13	7½ ,,	,, ,,	32 × 40
13	8½ ,,	,, ,,	39
36	11 ,,	,, ,,	Sporozoites in salivary glands
17	12½ ,,	,, ,,	30 (No sporozoites in salivary glands)

Measurements were made from fresh preparations in 10 per cent. formalin-saline under cover-slip with ocular micrometer (Zeiss).

The zygotes, as will be seen from the foregoing table, generally assume an ovoidal shape, one diameter being a little longer than the other. As they increase in size they become more vesicular, the periphery assumes a definite rim-like contour, and the pigment becomes more and more scattered, discrete, and ultimately, inconspicuous. During the first sixty hours the pigment is tightly clumped, but as the zygote becomes larger and vesicular, the pigment usually spreads out in lines or belts, sometimes in small clumps, or occasionally scattered and discrete. In Experiment No. 38 a specimen of *Ce. albimana* contained about fifty malignant

tertian zygotes, three and a half days old (12 × 12 μ), in its mid-gut; the pigment was in the form of linear rods, and almost every zygote had a zonal arrangement of its pigment. The zone of pigment was made up of rods, end to end, each rod separated by a small space; sometimes the zonal pigment was concentric with the periphery of the zygote, sometimes at right-angles to the plane presented to the observer, but apparently always peripheral.

In Experiment No. 33, a specimen of *Ce. albimana* contained twelve to fifteen malignant tertian zygotes, three and three-quarter to four and three-quarter days old, 12 × 13·5 μ in size, mostly oval in outline. The pigment was bronze in colour, and in clumps, never in lines or belts. In experiment No. 36 a specimen of *Ce. albimana* contained malignant tertian zygotes five days old, 21 μ in diameter, with the pigment present in clumps. On counting the number of pigment rods in a zygote, and in crescents, it is evident that conjugation of gametes does not occur, for there is the same amount of pigment in each instance. In Experiment No. 42, a specimen of *Ce. albimana* contained several malignant tertian zygotes of three ages (three bitings from the same patient). Five or six zygotes were between one and a half and two and a half days old, and their pigment, of course, tightly clumped. One zygote, 21 μ in diameter, was globular and contained three clumps of pigment, while in several others of the same age the zygotes were oviform and the rods of pigment were arranged in pairs, scattered irregularly throughout the zygote, each one containing thirteen or fourteen rods of pigment.

In most of the zygotes, excepting the very young or very old forms, the pigment was arranged in lines or belts, but not uncommonly zygotes were seen of equal age in the same specimen, some with belts of pigment, and some having it in clumps.

The larger zygotes, containing sporoblasts, showed very little pigment. This is collected in one clump, usually near the periphery. The pigment is probably not destroyed nor extruded, but is partly obscured by the greater size of the zygote.

Some of the smallest zygotes contained dancing pigment, and in the larger zygote, with zones of linear pigment, the zone could be seen to change its position very slowly, but in these instances the pigment was not dancing.

When the zygotes reach the size of 39 × 45 μ, or 45 × 57 μ (seven to eight and a half days) a fine reticulum could be seen outlining the sporoblastic chamber; while others were dotted throughout with coarse round granules, the sporoblasts.

The capsule of the zygote ruptures about the eleventh day, the sporozoites making their way to the salivary glands, and leaving the collapsed, wrinkled, disc-shaped envelopes behind in the outer layer of the mid-gut wall. The mechanism of the passage of the sporozoites into the salivary glands is not known, but these glands are more or less filled with sporozoites after the eleventh day.

The simple tertian zygote differs from the malignant tertian; first in the rapidity of its development, attaining a slightly greater size in a given time. In the younger zygotes the pigment is arranged in clumps or lines and slowly changes its position. The pigment may be clumped and dancing within vacuolated spaces of the zygote. In Experiment No. 16, tertian zygotes in *Ce. albimana* are 12 to 16·5 μ in diameter four to four and a half days after biting. The capsule of the zygote was well defined, but the zygote was apparently slightly larger than a malignant tertian zygote of the same age, and its cytoplasm was more coarsely granular than that of the malignant tertian parasite. The pigment is coarse and in clumps, not lines, and is not motile. In another specimen of *Ce. albimana*, in the same experiment, the pigment was arranged in two lines near the periphery of the zygote. Nine days after the first biting, one *Ce. albimana* with half mature ova, contained six to eight zygotes, full of faintly refractile, equally sized sporoblasts, 3 μ in diameter. These zygotes were 48 μ in diameter. Four or five zygotes were seen partly projecting from the outer wall of the gut. They were 48 by 54 μ and 58·5 by 66 μ. No pigment could be detected, but here and there faint clusters in groups, having a linear arrangement, indicating the development of sporozoites from sporoblasts. In this experiment the zygotes nearly reached maturity in nine days.

In Experiment No. 18, simple tertian sporozoites were found in only one of five acini of the salivary glands in a specimen of *Ce. albimana* eleven and a half days after the first biting. Four other acini were in plain view, but were entirely devoid of sporozoites. The sporozoites in the infected acinus were distributed in the lumen of the duct and in several of the distended vesicular epithelial cells, several

hundred being present, their long axis being generally parallel with the duct.

In Experiment No. 36, malignant tertian sporozoites were found after the eleventh day in the red-stained hyaline salivary acini on both sides of the body in large numbers. Very few in the faintly stained, larger vacuolated acini, and none in the acini containing colloid globules; but the duct of the latter contained sporozoites, which were generally matted together.

Table showing data in relation to infected and non-infected mosquitos:—

Experiment No.	No. and species of mosquito	Type of parasite	No. of gametes	Dissected: days after biting	No. of zygotes present	Controlled by *Ce. albimana*	Date of experiment	No. of patient	Ova developed
2	1 M.*	?	5	3	0	No	Oct. 13	No. 9 18 B.	?
3	1 A.	E.A.†	10	3½	0	Yes	,, 19	47496	No
3	1 A.	E.A.	10	3½	5	Yes	,, 19	47496	Yes
3	1 A.	E.A.	10	4½	0	Yes	,, 19	47496	No
3	1 A.	E.A.	10	4½	0	Yes	, 19	47496	No
4	1 P.	E.A.	14 + −	5	5+ −	No	,, 27	48132	No
4	1 P.	E.A.	24 + −	5	31	No	,, 27	48132	No
5	1 M.	E.A.	3·5	4½	0	No	Nov. 10	48509	No
5	1 M	E.A.	3·5	4½	0	No	,, 10	48509	No
5	1 M	E.A.	3·5	4½	0	No	,, 10	48509	No
5	1 M.	E.A.	3·5	4½	0	No	,, 10	48509	No
6	1 M.	E·A.	16	3½	0	Yes	,, 11	48987	Yes
6	1 M.	E.A.	16	3½	0	Yes	,, 11	48987	No
6	1 M.	E.A.	16	3½	0	Yes	,, 11	48987	No
9	1 P.	E.A.	27	2½	0	No	,, 24	48987	No
9	1 P.	E.A.	27	2½	6 or 7	No	,, 24	48987	No
10	1 A.	E.A.	12	4+ −	Numerous	Yes	,, 27	48987	No
11	1 A.	E.A.	8	7	0	Yes	,, 30	48987	No
11	1 A.	E.A.	8	7	Present	Yes	,, 30	48987	No
11	1 A.	E.A.	8	7	168	Yes	,, 30	48987	No
12	1 P.	E.A.	8	2½	0	Yes	,, 30	48987	No
13	1 P.	E.A.	20	3½	0	Yes	Dec. 4	48987	No
13	1 P.	E.A.	20	3½	6 or 7	Yes	,, 4	48987	No
13	1 A.	E.A.	20	7½	3	Yes	,, 4	48987	Slightly
13	1 A.	E.A.	20	8½	Numerous	Yes	,, 4	48987	No
13	1 A.	E.A.	20	8½	Numerous	Yes	,, 4	48987	No
16	1 A.	Tert.	3+	4	6 or 8	Yes	,, 21	51147	No
16	1 A.	,,	3+	4½	20+ −	Yes	,, 21	51147	No
16	1 A.	,,	3+	8½	6 or 8	Yes	,, 21	51147	Yes
16	1 A.	,,	3+	8½	4 or 5	Yes	,, 21	51147	?
18	1 A.	,,	3	11½	1	Yes	,, 27	51431	Yes
32	1 A.	,,	17 + −	4¾	2	Yes	Jan. 25	53343	Yes
32	1 A.	,,	17 + −	4¾	0	Yes	,, 25	53343	Yes
32	1 Ag.	,,	17 + −	4¾	0	Yes	,, 25	53343	Yes
32	1 P.	,,	17 + −	4¾	0	Yes	,, 25	53343	Partly
32	1 P.	,,	17 + −	4¾	0	Yes	,, 25	53343	,,

Experiment No.	No. and species of mosquito	Type of parasite	No. of gametes	Dissected: days after biting	No. of zygotes present	Controlled by *Ce. albimana*	Date of experiment	No. of patient	Ova developed
32	1 P.	Tert.	17 + −	4¾	0	Yes	Jan. 25	53343	Partly
32	1 P.	,,	17 + −	4¾	0	Yes	,, 25	53343	Yes
33	1 A.	E.A.	11	4¾	12-15	Yes	,, 26	H.G.	Yes
33	1 A.	E.A.	11	4¾	12-15	Yes	,, 26	H.G.	Yes
34	1 P.	E.A.	4	4½	0	No	,, 27	H.G.	No
35	1 Ag.	E.A.	8	2½	0	No	,, 28	53499	No
36	1 A.	E.A.	27	5	Present	Yes	,, 30	53499	Yes
36	1 P.	E.A.	27	5	0	Yes	,, 30	53499	No
36	1 P.	E.A.	27	6	0	Yes	,, 30	53499	Yes
36	1 P.	E.A.	27	6	0	Yes	,, 30	53499	Yes
36	1 A.	E.A.	27	11	Present	Yes	,, 30	53499	Yes
36	1 A.	E.A.	27	13½	,,	Yes	,, 30	53499	—
37	1 P.	E.A.	29	3	0	Yes	Feb. 1	53742	No
37	1 P.	E.A.	29	3	0	Yes	,, 1	53742	No
37	1 P.	E.A.	29	3½	0	Yes	,, 1	53742	Yes
37	1 P.	E.A.	29	3½	0	Yes	,, 1	53742	Slightly
37	1 P.	E.A.	29	3½	0	Yes	,, 1	53742	,,
38	1 A.	E.A.	29	2¾	Present	Yes	,, 1	53742	No
38	1 A.	E.A.	29	3½	Many	Yes	,, 1	53742	Slightly
38	1 A.	E.A.	29	6¾	Numerous	Yes	,, 1	53742	Yes
38	1 P.	E.A.	29	11½	0	Yes	,, 1	53742	No
38	1 A.	E.A.	29	11½	Many	Yes	,, 1	53742	Slightly
41	1 A.	E.A.	92	3½	Numerous	Yes	,, 5	53937	Yes
41	1 A.	E.A.	92	8½	?	Yes	,, 5	53937	?
41	1 A.	E.A.	92	10½	—	Yes	,, 5	53837	—
44	1 P.	E.A.	3·5	1½	0	No	,, 14	53937	No
44	1 M.	E.A.	3·5	2¾	0	No	,, 14	53937	No
44	1 M.	E.A.	3·5	4½	0	No	,, 14	53937	Yes
44	1 M.	E.A.	3·5	4½	0	No	,, 14	53937	No
44	1 P.	E.A.	3·5	4½	0	No	,, 14	53937	Slightly
46	1 A.	E.A.	2	1½	0	Yes	,, 17	53937	No
47	1 P.	E.A.	½	3½	0	Yes	,, 19	53937	No
47	1 P.	E.A.	½	3½	0	Yes	,, 19	53937	No
47	1 A.	E.A.	½	3½	0	Yes	,, 19	53937	Slightly
47	1 A.	E.A.	½	3½	?	Yes	,, 19	53937	Yes
47	1 A.	E.A.	½	3½	No	Yes	,, 19	53937	Yes
47	1 A.	E.A.	½	3¾	3	Yes	,, 19	53937	No
47	1 A.	E.A.	½	3¾	4	Yes	,, 19	53937	No
47	1 P.	E.A.	½	3¾	0	Yes	,, 19	53937	No
47	1 M.	E.A.	½	3¾	0	Yes	,, 19	53937	No
47	1 A.	E.A.	½	3¾	0	Yes	,, 19	53937	Slightly
47	1 M.	E.A.	½	3¾	0	Yes	,, 19	53937	No
47	1 P.	E.A.	½	3¾	0	Yes	,, 19	53937	No
47	1 P.	E.A.	½	3¾	0	Yes	,, 19	53937	No
47	1 P.	E.A.	½	3¾	0	Yes	,, 19	53937	No
42	1 A.	E.A.	10	1½	0	Yes	,, 6	53742	No
42	1 A.	E.A.	10	1½	0	Yes	,, 6	53742	No
42	1 P.	E.A.	10	1½	0	Yes	,, 6	53742	No
42	1 A.	E.A.	10	3¾	Several	Yes	,, 6	53742	Yes
42	1 M.	E.A.	10	3¾	0	Yes	,, 6	53742	Yes
42	1 A.	E.A.	10	4½	Present	Yes	,, 6	53742	Yes
42	1 A.	E.A.	10	4½	,,	Yes	,, 6	53742	Yes
42	1 A.	E.A.	10	5¾	Many	Yes	,, 6	53742	Yes
42	1 A.	E.A.	10	5¾	,,	Yes	,, 6	53742	Yes
42	1 A.	E.A.	10	5¾	3 or 4	Yes	,, 6	53742	Yes

Experiment No.	No. and species of mosquito	Type of parasite	No. of gametes	Dissected: days after biting	No. of zygotes present	Controlled by *Ce. albimana*	Date of experiment	No. of patient	Ova developed
42	1 A.	E.A.	10	$5\frac{3}{4}$	3 or 4	Yes	Feb. 6	53742	No
43	1 A.	E.A.	5	$2\frac{3}{4}$	5	Yes	,, 8	53742	No
43	1 M.	E.A.	5	$2\frac{3}{4}$	0	Yes	,, 8	53742	No
47	1 P.	E.A.	$\frac{1}{2}$	$5\frac{1}{2}$	0	Yes	,, 19	53937	No
47	1 M.	E.A.	$\frac{1}{2}$	$5\frac{1}{2}$	0	Yes	,, 19	53937	No
47	1 Ag.	E.A.	$\frac{1}{2}$	$5\frac{1}{2}$	0	Yes	,, 19	53937	No
47	1 Ag.	E.A.	$\frac{1}{2}$	$5\frac{1}{2}$	0	Yes	,, 19	53937	No
47	1 M.	E.A.	$\frac{1}{2}$	$5\frac{1}{2}$	0	Yes	,, 19	53937	No
202	1 P.	?	0?	4	0	No	Aug. 24	Bed 90	Yes
202	1 T.	?	0?	4	0	No	,, 24	Bed 90	Yes
204	1 A.	E.A.	5 + −	3	40	Yes	,, 28	63472	Yes
204	1 T.	E.A.	5 + −	4	Many	Yes	,, 28	63472	No
204	1 A.	E.A.	5 + −	9	Many	Yes	,, 28	63472	Yes
209	1 T.	E.A.	5 + −	2	20	Yes	Oct. 5	65343	Yes
209	1 T.	E.A.	5 + −	2	22	Yes	,, 5	65343	Yes

*A. = *albimana*
M. = *malefactor*
T. = *tarsimaculata*
P. = *pseudopunctipennis*
Ag. = *argyrotarsis*

†E.A. = malignant tertian
Tert. = simple tertian

Notes and Conclusions from the foregoing Table

Species	Number	Infected	Percentage infected
Arr. (?) malefactor	17	0	0·0
A. pseudopunctipennis	31	4	12·9
Ce. albimana	48+2	34+2	70·2
**Ce. argyrotarsis*	4	0	0·0
Ce. tarsimaculata...	5	3	60·0

* A naturally-infected specimen of this species has been found in barracks.

Out of several hundred mosquitos used in the biting experiments 107 gave satisfactory dissections, or paraffin sections, and it was determined that:—

70·2 per cent. of *Ce. albimana* became infected;
60 per cent. of *Ce. (?) tarsimaculata* became infected; and
12·9 per cent. of *A. pseudopunctipennis* became infected;

while none of *Arr. (?) malefactor* became infected, although several were placed in jars with *Ce. albimana* and bit at the same time persons from whom the specimens of *Ce. albimana* became infected.

It is concluded from this series of experiments that *Ce. albimana*, the common white hind-footed mosquito—an extremely hardy, rapidly developing, adaptable mosquito—is the transmitter of malignant tertian and of simple tertian malarial fever in the Canal Zone at this time. Specimens of this species infected with simple tertian parasites became infective between nine and eleven and a half days after the first feeding. When infected by malignant tertian parasites, sporozoites appeared in the salivary glands as early as the eleventh day in some mosquitos, and later than twelve and a half days in others.

Ce. (?) tarsimaculata appears to be as susceptible to the malignant tertian parasite as *Ce. albimana*, and no doubt if a favourable opportunity had presented it would have been found that *Ce. (?) tarsimaculata* would have been equally susceptible to tertian malaria.

A. pseudopunctipennis is only slightly concerned in the transmission of malarial fever, if at all, not only from the fact that only four out of thirty-one mosquitos under the most favourable conditions became infected, but from the additional fact that relatively few specimens are taken in quarters at this time.

Arr. (?) malefactor is not concerned in the transmission of malarial fever in the Canal Zone at this time.

Out of forty-one mosquitos containing malarial zygotes, seventeen showed development of ovaries or ova. A few of these, however, might have oviposited before examination.

Out of sixty negative bitings, i.e. where mosquitos of any of the species bit and failed to become infected, twenty showed development of ova; and to take the known susceptible species, *Ce. albimana* and *Ce. (?) tarsimaculata*, out of thirteen *Ce. albimana* and *Ce. (?) tarsimaculata* which failed to become infected six showed development of ova.

It would, therefore, appear that fecundation and development of ova are not necessary for the development of zygotes.

Under the most apparently favourable circumstances one or two out of the lot of susceptible Anophelines will fail to become infected, and these, no doubt, possess an active immunity toward the malarial parasite.

LIMITS OF INFECTIOUSNESS OF MAN

During the progress of the experiments it was noticed that patients were discharged after their temperature had become normal and when their peripheral blood occasionally contained more than a sufficient number of gametes to infect susceptible mosquitos. In order that a recommendation might be made for the continued treatment of these persons, it was necessary to determine, if possible, the limits of infectiousness of such individuals.

Several experiments were carried out, in which *Ce. albimana* bit patients whose gametes per leucocytes had been determined. These mosquitos were given but one blood feeding and fed subsequently on dates and raisins and then dissected. The limits were determined as being near one gamete per 500 leucocytes, or twelve gametes per mm.^{3}; but it must be understood that several factors are concerned in infections, such as number and phagocytic power of leucocytes; immunity of mosquitos, racial and individual; probable reaction of gut contents, as acid bacterial products or those from yeasts, may be inimical to the gametes; and again proportion of ♀ to ♂ gametes plays some part, besides the number of gametes ingested. In Experiments 20 and 28 patients' blood was rich in crescents which flagellated *in vitro*, and in the mosquito's stomach, yet mosquitos never could be infected from the patients.

Persons with more than twelve gametes per mm.^{3} must be regarded as gamete carriers, and, of course, should not be discharged from hospital nor should treatment be discontinued until gametes have been reduced well below the limits of infectiousness. This destruction and prevention of the development of the sexual forms of the parasite in man is a matter generally overlooked, but is of the greatest importance in delimiting malaria, and it may be accomplished by appropriate quinine treatment of all gamete carriers; by quinine treatment to destroy latent malaria, and by periodical blood examination of labourers and others in quarters where there is a high malarial rate. For the detection of gamete carriers and latent malaria, in order to carry out appropriate treatment, 30 grains of quinine sulphate in solution daily is an efficient dosage for the purpose required.

EXPERIMENT No. 47.—February 19, 1909, 8 p.m., jar containing *Ce. albimana, A. pseudopunctipennis* and *Arr. (?) malefactor* was applied to No. 53,937, who was receiving 30 grains of quinine daily when his blood contained one crescent to 200 leucocytes = approximately + 30 gametes per mm.³. The mosquitos were given but one blood feeding and subsequently fed on raisins and dates.

Upon dissection on February 23-5 :—

9 *A. pseudopunctipennis* were not infected
4 *Arr. (?) malefactor* ,, ,,
2 *Ce. argyrotarsis* ,, ,,
3 *Ce. albimana* ,, ,, while
1 *Ce. albimana* contained three zygotes, and
1 *Ce. albimana* contained four zygotes.

EXPERIMENT No. 31.—January 25, 1909, a jar containing *A. pseudopunctipennis, Ce. argyrotarsis* and *Ce. albimana* were fed from 53343, tertian malaria fever, whose blood contained four gametes per 100 leucocytes.

Upon dissection, January 30 :—

3. *A. pseudopunctipennis* were not infected;
1 *Ce. argyrotarsis* was not infected;
1 *Ce. albimana* was not infected; while
1 *Ce. albimana* contained two zygotes.

NOTES ON THE BIONOMICS OF SOME OF THE ANOPHELINES STUDIED

The period of incubation of the ova of *Ce. albimana, A. pseudopunctipennis* and *Arr. (?) malefactor* was estimated as about thirty-six hours under the laboratory conditions, the temperature of the air and water ranging between 78° and 82° daily, an eastern window exposure with direct sunlight for three or four hours in the morning, and diffused sunlight the remainder of the day. The eggs are laid in the geometrical patterns usually seen, and at first are creamy-white colour, becoming in a few hours quite black, with white lateral air chambers.

The larval period varies with the species, food, efficient temperature, sunlight and environment; for example, ova of *Arr. (?) malefactor* and *Ce. albimana* of about the same age were exposed in the same breeding tank to an identical environment, food, water, air, and sunlight; but when *Ce. albimana* had pupated the larvae of *Arr. (?) malefactor* were only half grown. In one experiment *Ce. albimana* larvae pupated within twelve days, while *Arr. (?) malefactor* required sixteen to twenty days. Subsequent examinations of water from *Arr. (?) malefactor* pools and from the intestinal tracts of *malefactor* larvae indicate that the latter

prefer shady pools in which chlorophyll-bearing algae, the chief food of *Ce. albimana* larvae, are relatively absent.

Ova of *Ce. albimana*, still creamy-white in colour, were placed in a breeding tank exposed to the morning sun on December 3; temperature of the water, 28·5 to 30° C. (78° to 82° F.). Of these ova five became larvae and pupated December 14-15. These pupae became imagines during the night of 16-17, making the period from ovum to imago about thirteen and a half days. Under these conditions they did not get as much sunlight as they would have received outside. Sunlight and the abundance of algae undoubtedly play a great part in the duration of the period of incubation. It should be added that these five mosquitos, two males and three females, were placed in a biting jar the morning they emerged—December 17, and that same evening each one of the three females bit and drew blood at once when applied to the arm of a patient. In this instance mosquitos bit when not more than twenty-hour hours old.

Into the same tank were placed sixty-eight larvae of *Arr. (?) malefactor* from ova laid December 3; one-third of the ova hatched the morning of December 5, the approximate age of the ova, or the period of incubation, was thirty-six hours. The tank was supplied with algae, spirogyra, and the water aerated. It was noticed very soon that the *malefactor* larvae did not mature as rapidly as the *albimana* larvae did; when specimens of the latter were full grown the *malefactor* were only one-third or half grown, and the *malefactor* larvae did not pupate until sixteen to twenty days after hatching.

HARDINESS OF *CE. ALBIMANA*

This mosquito is well fitted for the purpose of transmitting malarial fever. It is the commonest species here at the present time, outnumbering all others, excepting, possibly, *A. pseudopunctipennis*, which latter species is not very hospitable to the malarial parasite.

It breeds in a great variety of locations; besides the customary pools and margins of streams, collections of rainwater, during the dry season it may be found in the stinking water of sewage streams, brackish marshes, running streams, meadows, muddy pools, old crab holes, and in shady *malefactor* pools and river margins.

It matures more rapidly than either *A. pseudopunctipennis* or *Arr. (?) malefactor.*

It outlives *pseudopunctipennis* and *malefactor,* in confinement at any rate, which is a proof of its ability to persist, for when these species are placed in one breeding jar *malefactor* dies quickly, next follow *pseudopunctipennis,* while specimens of *albimana* survive for days longer.

DURATION OF LIFE OF MALES AND FEMALES IN CAPTIVITY

Male specimens of *A. pseudopunctipennis* which have been kept in breeding jars with females, and supplied with raisins, dates and water, have lived for eighteen days. Male specimens of *Ce. albimana* have lived twelve and a half days. On the other hand, a virgin specimen of *Stegomyia calopus* has lived for one hundred and ten days.

When virgin Anophelines have been given one or two blood meals two or three days after emerging they have lived as long as sixteen days. This is in rather striking contrast with *Stegomyia calopus,* which under similar conditions lives months to the Anophelines weeks. Several specimens of *S. calopus* virgins still under observation have lived for several weeks, and have oviposited as late as sixty days after emerging, though never in contact with males.

MUSICAL NOTE OF MOSQUITOS

The characteristic musical note of Anophelines is caused by the vibration of the proboscis, as the following observation indicates:—

A specimen of *Arr. (?) malefactor* was badly wet and sprawled; upon placing her upon a piece of filter paper and touching or approaching her proboscis the latter vibrated visibly, and emitted the characteristic high-pitched note; the wings were at rest, being stuck to the paper. This was verified again and again. Later I picked up a slightly water-sprawled, infected mosquito for dissection and held it over a few drops of chloroform; both wings were seen to vibrate rapidly as in flight, but noiselessly, while holding the mosquito by the last abdominal segment and touching one wing at its tip the opposite wing would immediately stop

vibrating. Upon releasing the wing, both would vibrate noiselessly as before. The noise of the mosquito is due, then, to the vibration of its proboscis, and the wing vibration is dependently and automatically co-ordinated.

RELATIVE VALUE OF DIFFERENT FOODS FOR ANOPHELINES

After trial with bananas, I found that raisins and dates with water furnished the best food for Anophelines in confinement. A greater number of males and females may be kept alive during the few days after emergence if fed with fresh bananas, raisins or dates; but females fed on this diet daily would not bite with alacrity. Mosquitos fed daily or on alternate days on human blood made better dissections after the digestion and evacuation of their meal than those fed on bananas. The former would be fairly free from yeast and bacteria, and the mid-gut and appendages would not disintegrate so rapidly after death. If the mosquitos were fed alternately on bananas and blood they would frequently die with an undigested hard mass of blood in the mid-gut, which must have been either impossible to digest or evacuate. The best method of feeding infected mosquitos would seem to be to feed two or three times on a patient favourable for infection, and subsequently with raisins, dates and water. Then, too, the acid contents of the mid-gut after banana feeding, with its fermentation, may interfere with the infection of mosquitos by malaria. In Experiments Nos. 20 to 28 a patient having at times sixty-seven crescents per one hundred leucocytes in his peripheral blood was bitten by four varieties of mosquitos, *Ce. albimana*, *Ce. argyrotarsis*, *A. pseudopunctipennis* and *Arr. (?) malefactor*, over a period of thirty-five days. During the intervals between biting, however, the mosquitos were fed on bananas, and none became infected.

IDENTIFICATION OF LARVAE

It must be evident that identification of Anopheline larvae in the field is of considerable importance, and in this region the malaria-transmitting Anophelines can be readily identified by certain anatomical characters. I have made no attempt to determine in

detail all the anatomical characteristics of Anopheline larvae of this region; that has been done for some species by Knab. The chief anatomical differentiating larval characters of the common Anophelines of this region are these:—

Ce. albimana or white-footed group	Palmate hairs on all abdominal segments and sometimes on postero-external angle of thorax	Antennae without a tuft of hairs
A. psendopunctipennis group	Palmate hairs on third, fourth, fifth, sixth, seventh abdominal segments, but none on the first and second. On the latter two, however, there is a rudimentary stalked tuft.	Antennae without a tuft of hairs.
Arr.(?) malefactor, or spotted-legged group	No palmate hairs on first and second abdominal segments, but palmate hairs on all remaining segments.	Antennae with a tuft of hairs.

These characters are very striking and sharply separate the groups, thus separating the malaria-transmitting *Ce. albimana* group from other varieties. With care it is frequently possible, even in muddy water, from an examination of the indentations of the surface film caused by the palmate hairs, to at once determine the presence or absence of members of the *albimana* group. In the latter group there is no break in the indented film, but in the two former groups there is a well defined non-indented break in the film, due to the lack of palmate hairs on the first and second abdominal segments.

The size of the palmate hairs on the postero-lateral angle of the thorax and the presence of these hairs on the thorax, first and second abdominal segments, is subject to some variation. It would seem that the white hind-footed group are undergoing some variation with regard to the size and location of these hairs and apparently they are becoming rudimentary or vestigial on the thorax and first abdominal segment.

FOOD OF ANOPHELINE LARVAE

The generally separate and distinct breeding places of *Arr. (?) malefactor* and *Ce. albimana*, for instance, naturally suggest that their food might also be different. Dissection of specimens of *A. pseudopunctipennis* and *Ce. albimana* in all instances discloses much green algae in the intestinal tract, while

specimens of *malefactor* usually contains much dark brown, unidentified vegetable fibres, brown organic débris, and conidia resembling those of *Pestalozia trunculata*, *Leptosporum bifurcatum*, *Paramoecia*, etc., *Rotifer vulgaris*. This indicates that *Ce. albimana* and *A. pseudopunctipennis* prefer sunny pools, while *Arr. (?) malefactor* prefers shady ones, where there is a relative absence of chorophyll-bearing forms. This is not intended as an absolute statement, because during the dry season, and in certain situations, *malefactor* and *albimana* will be found together in the same streams or pools, but it indicates certain different tendencies in the respective species.

BLOOD FEEDING NECESSARY FOR ANOPHELINES

A blood meal seems to be necessary for the development of the ova of Anophelines. In Experiment No. 40, to determine this point, male and females, *Ce. albimana* and *A. pseudopunctipennis* were placed in a breeding jar and fed on vegetable food and water daily, but they received no blood meals. Upon dissection of females as they died, none showed any development of the ovaries.

PARTHENOGENESIS

If, however, there be given one blood meal the ova may develop even in virgins kept out of contact with males. In the latter instance (with *Stegomyia calopus*) the ova have never developed into larvae.

Experiment.—Virgin Anophelines bred out from single isolated pupae were transferred to one jar entirely out of contact with males. There were three *Ce. albimana* and two *A. pseudopunctipennis*. When applied to the arm all the *Ce. albimana* drew blood; neither of the *A. pseudopunctipennis* would bite. The following day one *A. pseudopunctipennis* died, but the remaining one, with two *Ce. albimana* bit again upon application to the arm. These were added to another jar of virgin females, *Ce. albimana* and *A. pseudopunctipennis*, most of which drew blood readily. Upon dissection none of the *A. pseudopunctipennis* showed any development of the ovaries. One *Ce. albimana*, about fourteen days old, contained ova 0·48 mm. long. The single spermathecae of the mosquitos were examined, and in no instance contained spermatozoa. The ova would not develop into larvae in water, and upon microscopic examination were found to contain finely granular food material, but no partly developed larvae, such as would be seen in fertile ova of this size.

A further experiment with *Stegomyia calopus* indicates that whereas ova may develop in size in the ovaries of unfecundated virgins, they are always sterile and never contain partly developed larvae, but granular, undifferentiated protoplasm or food material.

A. November 11 a jar containing five female *Stegomyia calopus* which had been separated individually as pupae, and always out of contact with males emerged November 9. They were applied to the arm and three bit and drew blood; they were not as voracious as Anophelines, but behaved with the caution and timidity characteristic of *Stegomyia*. After fourteen days these mosquitos had not oviposited. Two were dissected and the spermathecae found to be free from spermatozoa, but their ova were well developed, 0·560 mm. in length, 0·184 mm. in width.

B. Bred out nineteen virgins, *Stegomyia calopus,* in the same manner, from isolated pupae. Upon applying to arm, sixteen out of nineteen bit and apparently drew blood, but there was no sensation of stinging. The next day when applied, only three mosquitos bit. These may have been the ones that did not bite the day before. Thirty-seven days after the first biting, eight of these virgins were living, the dead ones having become water-sprawled. Forty black ova were found in the water dish this morning.

41 days after the first biting six ova were found in the dish;

54 days after the first feeding one dead female was found, and upon dissection contained no ova;

69 days after the first feeding a dead female was found to contain twenty well-developed ova, 0·560 mm. in length;

61 days after first feeding forty-three black ova were found in the dish, completely developed, 0·720 mm. in length, 0·240 mm. in width;

but none of these ova developed later into larvae. One hundred and four days after the first feeding the remaining female is still living.

EFFECT OF SALT AND SEA WATER ON ANOPHELINE LARVAE

In general, the effect of an irritating, toxic, or otherwise unusual fluid on mosquito larvae is to hasten pupation. A number of experiments were tried with sea water, salt water and solutions of the heavy metals, and in most instances in the more concentrated solutions, when the larvae were not killed within twenty-four hours, they pupated, and occasionally the period of pupation was shortened; so that if, for instance, in a district sea water were used as a larvacide the first effect would be to hasten pupation and thus increase the number of Anophelines in the district, and if later the sea water became diluted by rain, several species of malaria-transmitting Anophelines might breed in it without difficulty, notably *Ce. albimana* and *Ce. tarsimaculata*. On this account sea water could not be used with any degree of success as a larvacide for Anophelines, except in large quantities and in certain locations.

CHLORINE CONTENTS OF NATURAL WATERS IN WHICH MOSQUITO LARVAE HAVE BEEN TAKEN, AND IN SOME INSTANCES BRED OUT

	Per cent. of Sodium Chloride
A. pseudopunctipennis	0·00165
Ce. albimana	1·93
Anopheles (Sp.)	0·65
Ce. albimana	1·165
Ce. albimana	0·255
Arr. (?) *malefactor*...	0·16
Ce. albimana	0·16
Arr. (?) *malefactor*	0·00002
Ce. albimana	0·00125
Ce. tarsimaculata	0·16
—	0·21
—	0·63
—	1·02
Ce. albimana	0·02
A. pseudopunctipennis	0·02
Stegomyia calopus	0·26
Culex (Sp.)	0·057
Aedes taeniorbynchus	2·20

Sea water taken from Panama Bay contained 3 per cent. of sodium chloride, a sample from Simon Bay (Atlantic) contained 3·17 per cent., so that it will be seen from the foregoing table that some Anophelines, under stress of circumstances, may breed in very brackish water.

EXPERIMENTS WITH LARVACIDES

A number of experiments were carried out for the purpose of obtaining a cheap and efficient preparation for destroying mosquito larvae. Crude petroleum oil was frequently too viscid to have a spreading power of the highest efficiency. When mixed with crude carbolic acid, however, its spreading powers were increased.

Much of the crude carbolic acid supplied had been found upon analysis to consist chiefly of inert neutral oils with a small proportion, 5 per cent. to 10 per cent., of tar acids, and as this crude acid was used extensively as a disinfectant, experiments were conducted for the purpose of utilising if possible this crude carbolic acid as a disinfectant and larvacide. It was found that crude carbolic acid, having a specific gravity not greater than 0·96 or 0·97 and containing about 20 per cent. of phenols or tar acids, when made into a soap with common resin and an alkali yielded a product which was an ideal larvacide, having

excellent diffusing and toxic powers, and at the same time it was a very efficient germicide. It diffused perfectly with water, forming a milky emulsion very destructive to mosquito larvae, and having a germicidal value of, or greater than, that of pure carbolic acid, or a Rideal-Walker co-efficient of one to two. In this way a very valuable larvacide and disinfectant, miscible with water, was produced from a very inferior disinfectant.

The larvacidal powers when tried with Culicine and Anopheline larvae varied slightly with the quality of the crude carbolic acid, but an average result is as follows:—

Dilution 1 to 1000—Culicine larvae, dead in 5 minutes.
Anopheline larvae, half grown, dead in 5 minutes.
Anopheline larvae, full grown, dead in 10 minutes.

Dilution 1 to 5000—Anopheline larvae, half and full grown, dead in 5 minutes.
Culicine larvae, half grown, dead in 3 minutes.

Dilution 1 to 10000—Culicine larvae, half grown, dead in 64 minutes.
Anopheline larvae, young, dead in 52 minutes.
Anopheline larvae, full grown, dead in 135 minutes.

Dilution 1 to 15000—Small Culicine larvae, dead in 32 minutes.
Anopheline larvae, full grown, dead in 123 minutes.

Anopheline larvae seem to be slightly more resistant than Culicine larvae, and all pupae are more resistant to the effects of the larvacide than larvae are.

EXPERIMENTS WITH AGENTS DESTRUCTIVE TO VEGETATION, GRASS AND ALGAE

A series of experiments was carried out with the larvacide, caustic soda, arsenic and copper sulphate as to the amounts necessary in pools and lagoons to prevent the growth of vegetation and to determine the value of the resulting solutions as larvacides. Bermuda grass in sod was made into artificial ponds in large glass moist-jars and flooded with 0·5 per cent. solution of caustic soda, copper sulphate and sulphuric acid. The sod was well soaked with the chemical solution, but the grass remained vigorous in each instance. The jars were undisturbed for a period of eighteen days, when a number of Culicine larvae were introduced into the solution of the artificial pools. The larvae were killed within twenty-four hours in the pools containing copper sulphate and sulphuric acid; but those in the pool

containing caustic soda remained alive several days. It was concluded from this that none of the above chemicals could be used to advantage in killing gross vegetable matter such as grasses, and none were of special value as larvacides.

An artificial pool as above was flooded with a 0·125 per cent. solution of sodium arsenite. All but three or four of the stalks of grass were killed and overgrown with mould, the wilting effect becoming apparent in forty-eight hours. After nine days, when the grass was quite dead, several Culicine larvae were introduced into the pool and were killed after one hour's exposure. The pool was twice flushed out to rid it of arsenic salt, but the grass showed no further signs of life at the end of thirty-five days. It was concluded from this that a 0·125 per cent. solution is a valuable agent in destroying gross vegetable forms such as grass, and the resulting water in the pool remained effective as a larvacide.

The common, green, filamentous algae, *Spirogyra* and Culicine larvae were introduced into small glass jars, containing various high dilutions of copper sulphate and sodium arsenite. The results of two series of experiments showed that copper sulphate in dilutions up to 1 part in 500,000 is inimical to the growth of this alga. They become greyish-green in colour, shrunken and lose their fresh and crisp appearance. As a larvacide, however, copper sulphate is not destructive in dilutions higher than 1 in 50,000 parts. Sodium arsenite, on the contrary, seems to stimulate the growth of these algae in all dilutions between 1 in 2,500 and 1 in 25,000,000, the algae remaining green and vigorous. As a larvacide, Culicine larvae were destroyed in sodium arsenite dilutions up to 1 in 100,000. The larvacidal powers of sodium arsenite solutions in contact with green algae seem to vary within wide limits, depending probably upon the power of the algae to take the arsenic salt out of solution into its own protoplasm, thus rendering the surrounding solution less larvacidal. It is concluded from this that copper sulphate is more efficient than sodium arsenite as an algacide in high dilutions, but the arsenic salt is a better larvacidal agent. These results are in keeping with our pharmacological knowledge of the effect of copper and arsenic salts in high dilutions on animal and vegetable protoplasm. It would seem, then, that when grass and algae in pools, without outlets, are to be destroyed, sodium arsenite

would be of considerable value for this purpose, and would continue to be efficient until washed or drained out and that copper sulphate is a valuable algacidal agent for the destruction of filamentous algae, as *Spirogyra*.

In experiments with the coal tar larvacide in laboratory tanks and under actual conditions the coal tar larvacide was found destructive to grass in dilutions of 10 per cent., the grass turning brown in two or three days and drying in five to six days. When *Spirogyra* was treated with the larvacide, dilutions of 1 to 2,500 were sufficient to kill, while dilutions of 1 to 5,000 and 1 to 10,000 greatly reduced its vigour.

COMPOSITION AND SIZE OF MESH OF WIRE SCREENING

Two extremely important factors in the use of wire screening for protection against mosquitos, are, first, the size of the mesh, and secondly, the chemical composition of the wire used. In regions where it is only necessary or desirable to protect against Anophelines, a No. 16 mesh screening (sixteen holes to the inch) would answer the purpose, and where, as in this region, it is necessary to protect against some of the smaller species, such as *Stegomyia calopus*, a No. 16 mesh would be practically safe, but not absolutely so. The following experiments were conducted to determine the varieties of mosquitos which would, under stress of circumstances, pass through a No. 16 mesh wire screening. Out of several hundred mosquitos eight common species were able to make their escape through a No. 16 mesh wire.

	Sex	No. of specimens escaped
Stegomyia calopus	Males	10
„ „	Females	6
Culex cubensis	Male	1
„ *rejector*	Male	1
„ *extricator*	Female	1
Aedes angustidittatus	Female	1
Uranotaenia lowii	Female	1

No specimens of *Ce. albimana* or *A. pseudopunctipennis* escaped through No. 16 wire mesh screen, although several hundred were tried. The methods adopted were as follows:—

A. A square wooden box, well ventilated, with fine crinoline gauze screening on two sides and glass on the other two sides, with a central replaceable partition, covered with No. 16 mesh wire screening, was constructed. Several dozen mosquitos at a time, of the above species were liberated on one side of the partition without food or water, and on the opposite side, close to the screen partition, were placed water, banana, candy, sugar and raisins as bait. Only three mosquitos out of several hundreds of several varieties passed through the No. 16 mesh partition under the conditions of the experiment. As the space including the mosquitos was about one-half of a cubic foot in volume, and as there were a few recesses in which the mosquitos could hide, an electric light bulb was hung in such a position at night that the mosquitos would be attracted by it, but this did not favour the passage of mosquitos through the screen. Tobacco fumes were passed into the mosquito compartment with a rubber bulb apparatus, and while this excited the mosquitos, did not cause any of them to escape through the screen. When a person's arm was introduced into the compartment close to the No. 16 mesh wire partition it did not induce the mosquitos to escape through the screening.

B. Next a lantern-chimney, covered on one side with fine mesh crinoline gauze and on the other side with a metal collar holding in place a piece of the No. 16 mesh wire screening, was partly filled with various mosquitos and placed near the same bait as before under a large glass bell jar. Eighteen mosquitos escaped from the chimney through the No. 16 mesh screening into the surrounding jar. The closer quarters and the absence of resting places in the chimney evidently favoured the escape of mosquitos through the wire screening. On one occasion, by passing a gust of air through the lantern chimney jar, a male *Culex* was helped through and escaped.

The conditions in the experiments were all rigid and more extreme than those under actual conditions where mosquitos are trying to enter a screened house from the open.

The chemical composition of various screening material used was investigated by Dr. R. W. Nauss, formerly of this Laboratory. Screening of excellent quality was compared with that which had deteriorated more or less rapidly, and analyses of screens and their incrustations made to determine the factors concerned in its corrosion.

In the investigation considerable attention was paid to the analyses of the efflorescence or incrustation formed on the screening for the determination of the constituents involved in the corrosion. The specimens presenting the highest degrees of deterioration furnished the largest amounts of incrustation. The deterioration of the screening is largely due to the presence of iron in the brass alloy, plus the influence of a hot, moist atmosphere.

Observations continued over a period of four years on screening made of copper and zinc with a composition nearly :—

Copper ...	84·92	89·94	84·83	88·59	95·85
Zinc	—	—	14·90	—	4·15
Iron	—	—	0·06	0·04	0·0

showed that these resist the corroding actions of a hot, moist climate much better than screening made of brass with an average composition of :—

Copper	65	
Zinc	34	
Iron	1	+ —

and it is concluded that screening intended for use in the tropics, exposed to heat and moisture, should have a high copper content, higher than brass, and be as free as possible from the presence of iron.

VALUE OF DAILY COLLECTION AND DESTRUCTION OF LIVE MOSQUITOS CAUGHT IN BARRACKS AND QUARTERS

Abundant material was received for observations on the value of this practice. The daily catch of mosquitos from barracks of various districts would be sent alive to the laboratory. The mosquitos were transferred to breeding jars and fed on dates or raisins until their intestinal tracts were free from blood. They were then killed and examined for zygotes or sporozoites. The species examined were: *Ce. albimana*, *Ce. tarsimaculata*, *Ce. argyrotarsis*, *Arr. (?) malefactor*, and *A. pseudopunctipennis*. A number of specimens of Culicines were also examined at this time. It is noteworthy, and speaks well for the practice of these daily killings, that but one naturally infected Anopheline was found, and that one a specimen of *Ce. argyrotarsis*.

After the first forty-three negative dissections no record was kept of the total number examined, but it was in the neighbourhood of five hundred.

EFFECT OF QUININE ON THE MALARIAL PARASITE IN (*A*) THE MOSQUITO AND (*B*) MAN

A. Nearly all the infecting experiments were conducted on patients who were receiving the routine ward treatment of quinine, grains 10, *ter. die*, in solution, so that apparently quinine in these quantities has no destructive or inhibitive effect on the parasites in the mosquito because the zygotes go on to maturity and sporozoites appear in the salivary glands in from nine to eleven days.

One experiment should be mentioned, however, because the patient received no quinine for several days before the mosquitos became infected and none during the experiment, so that the mosquitos never received any quinine. One *Ce. albimana* contained the rather large number of one hundred and sixty-eight zygotes upon dissection. It should be mentioned as well that from this patient two *A. pseudopunctipennis* became infected. It may be that quinine has a slight inhibitory effect on the parasite in the mosquito's mid-gut.

B. The following tables show the gradual but steady decrease in the number of gametes in the peripheral blood during the administration of quinine, grains 10, *ter. die*, in solution, and one table shows the effect of withholding quinine.

The differential leucocyte counts are tabulated as well, and in these the relative increase in mononuclear elements—lymphocytes, intermediate, and large mononuclear cell—the latter being the chief circulating phagocytic cell in malaria.

As indicative of blood regeneration and the secondary effect on the homopoietic organs, it is interesting to note the increase in the eosinophiles.

CHART 51499

	Dec. 30	Dec. 31	Jan. 2	Jan. 5	Jan. 6	Jan. 8	Jan. 13	Jan. 15	Jan. 23	Feb. 1
Polymorphonuclear	68	65	75	81	65	61·5	65	51	53	66
Large mononuclear	4	16	6	5	6	13·5	13	11	9·5	2·5
Lymphocyte	20	13	13	11	20	16·5	11	14	18	11·5
Large lymphocyte—Intermediate	—	—	—	—	—	—	—	8	4	7·5
Eosinophile	7	5	5	2	8	7	11	14	14·5	12·5
Mast	1	1	1	1	1	1·5	0	2	1	0
Crescents	67	42	76	46	40	15·5	9	5	0·5	0

The table given above shows the rate of diminution in the number of gametes (crescents) by the administration of quinine, grains 10, *ter. die*, in a Spaniard, 60 years of age, on Isthmus twenty months, whose blood contained numerous crescents but no young forms, and whose temperature was 97° F. continuously. It also shows the degree of change in the proportion of eosinophiles, of increase in mast cells, very slight lymphocytosis and polymorphonuclear decrease.

Compare this with the following:—Case 48,987 of malignant tertian malarial fever, from whom quinine was withheld for twenty-four days. Spaniard, on the Isthmus three months, temperature normal on admission.

1908.	Nov. 11	Nov. 24	Nov. 27	Nov. 30	Dec. 4
Polymorphonuclear ...	86	53	62	42	44
Large mononuclear ...	2	3	22	11	16
Lymphocyte	9	39	14	46	38
Large lymphocyte ...	0	2	2	0	0
Eosinophile	2	3	0	1	2
Mast	1	0	0	0	0
Crescents	16	27	12	8	20

In this case the continuance of the gametes in the peripheral blood is striking. The polymorphonuclear decrease and the lymphocytosis should be noted. This patient received no quinine during the period between November 11 and December 6. Young malignant tertian forms were always present in his blood with gametes. His temperature was irregular, and irregularly quotidian in character.

53,937: Spaniard, on the Isthmus twenty months, temperature normal, blood contained on admission many crescents but no young parasites; quinine, grains 10, *ter. die*, with Fowler's solution, gtts. 5.

CHART NO. 53937

	Feb. 5	Feb. 6	Feb. 8	Feb. 9	Feb. 11	Feb. 14	Feb. 15	Feb. 16	Feb. 17	Feb. 19
Polymorphonuclear ...	69	41	41	42	40	52	33	40	39	52·5
Large mononuclear ...	8	33	20	11	19	11	20	18	23	7·5
Lymphocytes	18	17	23	34	28	22·5	24	25	23	23
Large lymphocytes ...	3	8	10	8	5	10·5	6	14	10	7
Mast	0	0	0	0	1	0·5	0·3	0	2	0·5
Eosinophile	2	1	6	5	7	3·5	9	3	3	9·5
Crescents	92	87	61	48	20	3·5	4	3	2	0·5

This table illustrates, as in the preceding one, the steady disappearance of crescents under quinine, grains 10, *ter. die*, also the variation in the proportions of leucocytes.

It should be said that the specimens of blood were always taken at 4.30 or 8.30 p.m., or about four hours after a meal.

53,742 : Spaniard, sixteen months on the Isthmus ; blood : malignant tertian rings, crescents, ovoides ; spleen enlarged to umbilicus. February 1, quinine, grains 10, *ter. die.* ; February 6, Fowler's solution, gtt. 5, *ter. die.*

History No. 53742

	Feb. 1	Feb. 2	Feb. 3	Feb. 5	Feb. 6	Feb. 8	Feb. 9	Feb. 11
Polymorphonuclear	56	48	36	44	56	42	43	42
Large mononuclear	17	22	16	15	12	18	25	27
Lymphocytes	15	21	27	24	8	18	14	12
Large lymphocytes	8	4	3	8	10	12	8	9
Eosinophile	3	5	15	7	13	9	10	9
Mast	1	0	2	1	1	1	0	1
Pigmented leucocytes	—	—	1	1	—	—	—	—
Crescents	29	22	29	14	10	5	5	2

Notes :—Blood, February 2, 6,500 leucocytes per mm.^{3}.
Blood, February 5, containing one phagocyted gamete.

The large mononuclear increase is striking.

M. L., Spaniard, malignant tertian malaria, returned and died. Autopsy, March 28, 1909. Quinine grains 10, *ter. die.*

	Dec. 26	Dec. 27	Dec. 28	Dec. 30	Dec. 31	Jan. 2	Jan. 13
Polymorphonuclear	48	65	60	64	70	65	45
Large mononuclear	13	6	5	9	3	12	5
Lymphocyte	34	25	32	14	22	14	34
Eosinophile	4	4	3	12	5	8	16
Mast	—	—	—	1	—	1	—
Pigmented leucocyte	1	—	—	—	—	—	—
Crescents	9	5	5	4	1	0	0

Note :—Poikilocytosis and basophilia of red blood corpuscles on January 13.

The following two records are taken from cases of simple tertian malaria receiving quinine, grains 10, *ter. die.*

History No. 51147

	Dec. 21	Dec. 23	Dec. 26
Polymorphonuclear	69	18	48
Large mononuclear	7	39	12
Lymphocyte	23	36	30
Mast	1	—	—
Eosinophile	0	7	10
Gametes	3	1	0

Quinine discontinued after an initial dose.

History No. 50792

	Dec. 14	Dec. 15	Dec. 17	Dec. 20
Polymorphonuclear	64	48	17	23·5
Mononuclear	19	17	28	24
Lymphocyte	7	15	45	41
Eosinophile	10	19	8	10
Mast	0	1	1	0·5
Pigmented leucocyte	0	0	1	0·5
Gametes	?	?	0	0

Quinine discontinued after an initial dose. Returned January 11 with malignant tertian malaria—seven crescents per hundred leucocytes.

Hospital No. 50782

	Dec. 14	Dec. 15	Dec. 17	Dec. 20	Jan. 13
Polymorphonuclear	47	40	20	47	42
Large mononuclear	34	34	39	13	11
Lymphocyte	16	26	37	37	42
Eosinophile	3	0	4	2	4
Mast	0	0	0	1	—
*Gametes	0	0	0	0	—

*None of seven *Ce. albimana* became infected from this case, indicating the absence of gametes.

In each of the above cases the rapid polymorphonuclear decrease and the equally rapid mononuclear increase should be noted.

The effect of quinine administration, then, is to make the gametes gradually disappear from the peripheral blood by the destruction of the young forms, the gametes being phagocyted by splenic and hepatic endothelium. It is concluded that quinine, grains 10, *ter. die*, in solution, will gradually reduce the sexual form of the parasite in man to a non-infective minimum in from a few days to a few weeks, depending on the severity of the infection.

In simple tertian malarial fever, gametes disappear from the peripheral blood within two or three days under quinine treatment, and generally disappear even when quinine is withheld, if the patient is at rest. There are never as many gametes in the peripheral blood in simple tertian as in malignant tertian malaria. As a consequence, one never finds as many simple tertian zygotes as malignant tertian zygotes in infected mosquitos.

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